INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ03/00130

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12N 5/06, A61K 35/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, CA, WPIDS: pig, porcine, sertoli, pancrea?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03 027270 A (DIATRANZ LIMITED) 3 April 2003 See in particular pages 9 and 11 and figure 1	1-3, 6-26
X	WO 02 32437 A (DIATRANZ LIMITED) 25 April 2002 See in particular pages 3, 4, 27 and 28	1-3, 6-28
X	Cameron DF et al (2001) "Formation of insulin-secreting, sertoli-enriched tissue constructs by microgravity coculture of isolated pig islets and rat sertoli cells" **In Vitro Cell Dev Biol - Animal 37**, 490-8. See whole document.	1-28

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 August 2003	21 AUG 2003
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **TERRY MOORE** Telephone No : (02) 6283 2632

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ03/00130

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	2002 32437	AU	2002 11122	EP	1333846

END OF ANNEX

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 484955	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/NZ2003/000130**	International Filing Date *(day/month/year)* 24 June 2003	Priority Date *(day/month/year)* 24 June 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C12N 5/06 A61K 35/39

Applicant

DIABCELL PTY LIMITED et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of . sheet(s).

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of the report
16 December 2004	28 February 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **ALISTAIR BESTOW** Telephone No. (02) 6283 2450

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ2003/000130

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **2, 3, 5 to 14** , as originally filed,
pages , filed with the demand,
pages **1 and 4** , received on **15 February 2005** with the letter of **14 February 2005**

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **15 to 17** , received on **15 February 2005** with the letter of **14 February 2005**

☒ the drawings, pages **1 to 3** , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **1 to 28**	**YES**
	Claims **none**	**NO**
Inventive step (IS)	Claims **1 to 28**	**YES**
	Claims **none**	**NO**
Industrial applicability (IA)	Claims **1 to 28**	**YES**
	Claims **none**	**NO**

2. Citations and explanations (Rule 70.7)

Claims 1 to 28 disclose a method of preparing aggregates of porcine pancreatic islets and porcine Sertoli cells that upon implantation into a recipient produce insulin *in vivo*, and products thereof.

The applicants disclose a method where the Sertoli cells after culturing for at least one day, are scraped over the islets to form aggregates. This step is not present in the prior art and it is not an obvious step for the formation of aggregates. Thus the methods and products disclosed in claims 1 to 28 are both novel and inventive.

"PORCINE ISLETS FOR XENOTRANSPLANTATION"

TECHNICAL FIELD

The invention relates to the use of porcine pancreatic islet cells for the treatment of diabetes. More particularly but not exclusively it relates to the use of porcine pancreatic islet cells with associated Sertoli cells for the treatment of diabetes by xenotransplantation.

BACKGROUND

Background and Rationale for Porcine Islet Cell Xenotransplantation.

Type 1 (insulin-dependent) diabetes mellitus is a common endocrine disorder that results in substantial morbidity and mortality, and has a major financial impact on individual patients and healthcare systems. Treatment with insulin, while life-saving, often does not provide sufficient control of blood glucose to prevent the life-shortening complications of the disease, and this has given rise to intensive research into better methods of achieving and sustaining normoglycaemia. Among the newer treatment strategies that have been proposed, transplantation of pancreatic β islet cells, obtained either from other humans or animals, has received the most attention worldwide. This is because islet cell transplantation can restore not only the insulin-secreting unit, but also the precise fine-tuning of insulin release in response to multiple neural and humoral signals arising within and beyond the islets of Langerhans.

As human islet cell transplantation (allotransplantation) is limited by the shortage of human islet tissue, the use of pig islet cells is currently viewed as the most promising alternative since:

(a) pig and human insulin have close structural and biological similarities;
(b) physiological glucose levels in pigs are similar to those in humans; and
(c) the supply of pig cells can be readily expanded by optimising the supply of donor animals.

The rationale for this treatment approach (termed 'xenotransplantation') is that the implanted porcine islets have the potential to mimic the normal physiological insulin response in type 1 diabetics, such that near-normal blood glucose levels may be achievable without insulin or with a reduced requirement for it. As a consequence, long-term diabetes complications may be

consent to the procedure includes consent to ongoing post-transplant microbiological monitoring.

OBJECT OF THE INVENTION

It is an object of the invention to provide a method of treatment of diabetes, and/or a means to aid treatment of diabetes which has improvements to, or provides an alternative from, the abovementioned methods and/or means.

STATEMENTS OF THE INVENTION

According to a first aspect of the invention there is provided a method of preparing aggregates of porcine pancreatic islets and porcine Sertoli cells capable upon implantation into a recipient, of producing insulin in vivo, including or comprising the steps of:

1) isolation of porcine islet cells from the pancreas of donor piglets;
2) isolation of porcine Sertoli cells from the testes of donor piglets;
3) culturing the Sertoli cells for at least 1 day;
4) addition of isolated porcine cells to the cultured Sertoli cells at a predetermined ratio;
5) co-culturing the islet cells and Sertoli cells for at least 1 day;
6) scraping the Sertoli cell layer over the islets to form aggregates; and
7) culturing the aggregates for up to 24 hours.

Preferably the combination is in a predetermining ratio from 1:20,000 (islet:Sertoli cells) to 1:100; more preferably the ratio is between 1:2,000 to 1:4,000.

Preferably the culturing step is over a time period between 3 to 7 days more preferably it is for 5 days.

Preferably the isolation of the islets is followed by purification of the islets.

Preferably the isolation and purification of the islets together comprise or include the steps of:

a) surgical removal,
b) collagenase digestion,
c) washing and culturing of the islets.

CLAIMS:

1. A method of preparing aggregates of porcine pancreatic islets and porcine Sertoli cells capable upon implantation into a recipient, of producing insulin in vivo, including or comprising the steps of:

1) isolation of porcine islet cells from the pancreas of donor piglets;

2) isolation of porcine Sertoli cells from the testes of donor piglets;

3) culturing the Sertoli cells for at least 1 day;

4) addition of isolated porcine cells to the cultured Sertoli cells at a predetermined ratio;

5) co-culturing the islet cells and Sertoli cells for at least 1 day;

6) scraping the Sertoli cell layer over the islets to form aggregates; and

7) culturing the aggregates for up to 24 hours.

2. A method of claim 1 wherein said aggregate is a combination of islet:sertoli cells in a predetermining ratio from 1:20,000 to 1:100;.

3. A method of claim 2 wherein said ratio is between 1:2,000 to 1:4,000.

4. A method of any one of the preceding claims wherein said co-culturing step 5) is over a time period between 3 to 7 days.

5. A method of claim 4 wherein the time period is for 5 days.

6. A method of any one of the preceding claims wherein said isolation of the islets is followed by purification of the islets.

7. A method of claim 6 wherein the isolation and purification of the islets together comprise or include the steps of:

a) surgical removal,

b) collagenase digestion,

c) washing and culturing of the islets.

8. A method of claim 7 wherein said collagenase digestion involves Liberase H and Xylocaine.

9. A method of any one of the preceding claims wherein said isolation of the Sertoli cells is followed by purification of the Sertoli cells.

10. A method of claim 9 wherein said isolation and purification of the Sertoli cells together comprise or include the steps of:

a) surgical removal,

b) digestion with trypsin, Dnase,

c) washing and culturing of the cells.

11. A method of any one of the preceding claims wherein the method further includes the additional step of:-

8) virological and microbiological testing and/or monitoring of the aggregates and/or components thereof.

12. A method of any one of the preceding claims wherein the method additionally or alternatively includes a prestep before step 1 of virological monitoring and/or testing of one or both of the islets and Sertoli cells.

13. A method of any one of the preceding claims wherein the method additionally or alternatively includes a pre-step of virological monitoring and/or testing of the piglet donors.

14. A method of any one of the preceding claims wherein said islets and Sertoli cells derive from the same herd or from the same donor piglet(s).

15. A method of claim 14 wherein the piglet(s) are about one week old donors.

16. A method of any one of the preceding claims wherein the piglet(s) are monitored and/or tested for infectious agents.

17. A method of any one of the preceding claims wherein said piglet(s) are from a New Zealand pig herd.

18. A method of any one of the preceding claims wherein the step of the formation of the aggregate additionally or alternatively includes the preservation of the original characteristics and/or native structure of the islets.

19. An aggregate of porcine islets with Sertoli cells prepared substantially according to a method of any one of claims 1 to 18.

20. A method of treating a patient suffering from diabetes mellitus comprising or including the steps of:

1) preparing one or more aggregates of porcine islets with Sertoli cells prepared substantially according to a method of any one of claims 1 to 18,

2) implanting or otherwise administering one or more aggregate to the patient.

21. A method of claim 20 wherein said step of implanting or administering the aggregate may be by:

- encapsulation of the aggregate in a suitable biocompatible material,
- confinement into a suitable device
- inclusion in a matrix preparation selected from gelatin, collagen, and natural carbohydrate polymers; and
- inclusion in plasma thrombin clot or an autologous plasma clot produced with allogeneic thrombin.

22. A method of claim 21 wherein the biocompatible material is a suitable alginate.

23. A method of any one of claims 21 to 22 wherein said device is a vascularized tube.

24. A device for implantation into a recipient suffering from diabetes mellitus, the device incorporating aggregates of porcine pancreatic islets and porcine Sertoli cells, the aggregates being, or possessing the characteristics of, the aggregates of claim 19.

25. A device of claim 24 wherein said device incorporating the aggregates may be one of:

- a suitable biocompatible material as a capsule;
- a vascularized tube;
- a matrix preparation comprising gelatin, collagen, or natural carbohydrate polymers.
- a plasma thrombin clot or an autologous plasma clot produced with allogeneic thrombin.

26. A device of claim 25 wherein said biocompatible material is a suitable alginate.

27. A method of preparing aggregates of porcine pancreatic islets and porcine Sertoli cells prepared substantially according to Figure 1.

28. An aggregate of porcine pancreatic islets and porcine Sertoli cells substantially as described herein and with reference to any one or more of Figures 1 to 5.